REMARKS

Applicants have amended claim 18 to refer to a method to identify a "fish with a gene mutation." Support for this amendment can be found throughout the specification, and for example, at pages 13, lines 19-22, page 14, lines 1-5, and at page 17, lines 6-8.

Applicants have further amended the claim 18, step c) to refer to "haploid eggs" that are "derived" from the F1 generation "female fish." Support for this amendment can be found, for example at page 29, lines 22-25.

Accordingly, the present amendments are supported by the specification and do not introduce new matter and their entry is respectfully requested. Applicants believe that the amendments to the claims clarify the method and at minimum reduce the issues for the appeal.

Turning now to the specific rejections by the examiner.

Claim Rejections Under 35 USC § 112

Claims 18-29 were again rejected under 35 USC § 112.

Applicants have amended the claims to refer to a fish with a mutant. Applicants further submit that the method of the present invention is complete without an actual identification of the specific nucleic acid sequence or mutation thereof that causes the "mutant phenotype."

The present invention includes two screening steps, first screening step is performed using screening of embryos, without the need for growing the whole fish to detect a cell cycle defect causing mutation in the embryo. Second screen is to screen for the fish **harboring** a mutation that results in changes in carcinogenesis. Using these screens one can identify fish that harbor mutations involved in carcinogenesis. The method produces fish that can be used in either reverse genetic screening to find a curative mutation to counter the carcinogenic mutation, or the mutant fish can be subjected to a small molecule screen to screen for molecules that either delay, prevent or cure the cell proliferation defect affecting carcinogenesis. All this screening can be **performed before cloning** of the phenotype causing the gene mutation, yet knowing, before cloning, that the underlying mutation is involved in both cell cycle and carcinogenesis.

Therefore, the invention provides an elegant and novel system, where the cloning of the genes and identification of mutations can be avoided or deterred until a cure for that specific defect has been identified using a mere phenotypic screening system. The method also provides a two step system, where the whole animal use is minimized to expedite the screening of mutations that are involved in carcinogenesis. Therefore, the method does not only **not** require the step of cloning the gene and detecting the mutation, rather, it purposefully **avoids** such step.

Examiner is further puzzled by the phrase "exposing eggs of said F1 generation to inactivated sperm." The inactivated sperm allows one to produce **haploid embryos.** Therefore, the eggs are referred to "non-fertilized eggs." Applicants have amended step c) to refer to "haploid eggs" to make this point and therefore respectfully submit that the claim now clearly defines the metes and bounds of the claim.

Applicants further submit, that the terms "mutagen" and "carcinogen" as used in the claim are clear from the context. Furthermore, applicants use the term "a mutagen" in step a) and "a carcinogen" in step f. Since applicants do not say "the" or "said" carcinogen, it should be clear to one reading the claim that the molecule that the fish is exposed to is meant to be carcinogenic in a sense of **adding additional stress** in step f) to the **already mutagenized** fish which mutagenesis was performed in step a).

In light of the amendments and arguments presented above, applicants therefore respectfully submit that the rejections under 35 USC § 112, second paragraph, should be withdrawn. The examiner is encouraged to contact the undersigned, if she would have a suggestion to a more specific language to describe the present method.

Claim Rejections Under 35 USC § 103(a)

Claims 18 and 19 were rejected as obvious.

Applicants refer to the previous Amendment and re-submit all the arguments presented therein. In addition, applicants submit the following:

The present invention is directed to a particular method which **combines two mutation screening systems** to identify fish that harbor mutations related to cell proliferation defects and

carcinogenesis. The method avoids the necessity of first cloning the gene before it is known that it plays a part in both cell proliferation and carcinogenesis. The method further avoids use of growing the fish into adulthood in the first screening phase to expedite the method and to make it more convenient for large scale screening and after the connection of both with cell cycle defect and then with carcinogenesis is established, allows cloning the gene and mutation in question.

None of the cited references suggests using this kind of dual screening for identifying fish with mutations related to carcinogenesis.

Cheng teaches haploid screen, in which a non-fertilized eggs are exposed to the UV-inactivated sperm. Contrary to examiner's interpretation, the eggs are not fertilized, because that would defeat the purpose of the **haploid** (i.e. one genome containing) screening. However, Cheng goes further and states at page 529, second column, lines 4-12, that this method "makes late embryonic phenotypes difficult or impossible to detect." The present method has come up with a system, where just this, later development of problems, such as carcinogenesis CAN be detected. This sentence, rather than teaching someone to use haploid screen in combination with any other screen, suggests that the haploid screen is **not optimal** for purposes of identifying genes that are associated with later development of phenotypes, such as tumors. Thus, if anything, **Cheng teaches against** using haploid screen to identify cancer related genes.

There is nothing in Cheng that directs one to combine the haploid screen with a second screen for carcinogenesis and thus allow cloning of genes only after knowledge has been acquired that the genes/gene mutations are indeed involved carcinogenesis by way of affecting cell proliferation.

Spitsbergen does not overcome the defect in Cheng. Spitsbergen does not teach a dual screening system. Spitsbergen teaches a carcinogenesis screen of a mutant fish, **any mutant fish,** in itself. There is nothing in Spitsbergen that teaches that the carcinogenesis screen can be made vastly "more targeted" mutation hunting method by **combining** the carcinogenesis screen to a haploid screen for cell cycle mutant fish, which essentially leads one to identify the gene mutation's consequences and relationship with cancer **before** proceeding through the tedious steps of cloning the gene. This is the method that applicants claim and teach. This method

allows only cloning genes that are already known, by the method, to be relevant to the carcinogenic phenotype. Spitsbergen does not teach subjecting a fish that has been determined to carry a cell cycle defect to a carcinogenesis screen.

Therefore, applicants submit that only a person with knowledge, i.e. with hindsight, of the present disclosure of this ingenious combination of methods to speed up the discovery of carcinogenesis related genes and mutations in a fish system, would think that the method is obvious. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claims 20, 21, 23, and 29, were rejected as being unpatentable over Cheng, Spitsbergen, in view of Driever and Alexander.

As shown above, and incorporated herein by reference, Cheng in combination with Spitsbergen does not teach the combination of methods. Neither Driever nor Alexamder overcome this defect.

Both of these references deal with identification of genes important in vertebrate development. Further, neither Cheng nor Alexander teach comparison of tumor formation frequency of mutagen exposed wildtype fish to that of a mutagen exposed mutant fish, and identifying the carcinogenesis associated gene solely on the basis of accelerated tumor formation without first having to clone the gene first. Therefore, the combined use of the haploid screen with the carcinogenesis screen to allow screening of vast amount of mutations and then cloning only the phenotypically relevant ones, is not taught by the combination of these references.

Therefore, applicants submit that the rejection of claims 18-21, 23, and 29 under 35 USC § 103(a) over Spitsbergen in view of Cheng and Driever and Alexander is improper and should be withdrawn.

Claims 22 and 24 were rejected under 35 USC § 103(a) as being unpatentable over Cheng, Spitsbergen, and Driever, in view of Epstein.

Applicants respectfully disagree. Cheng, Spitsbergen, Driever were discussed above and the arguments are incorporated herein. Epstein merely teaches antisense oligonucleotides and their use as cell proliferation markers but does not in any way teach or suggest use of such probes in a zebrafish nor does Epstein teach a screen for novel carcinogenesis associated genes

using zebrafish haploid screen followed by carcinogenesis assay. Epstein does not teach the step of comparing the frequency of tumor formation between a mutagen exposed wildtype fish and mutagen exposed mutant fish and thereby determining mutations associated with carcinogenesis without cloning the gene first. Therefore, Epstein does not overcome the deficiency in Spitsbergen, Driever, Cheng and Alexander as described above, the discussion of which is incorporated herein by reference. Consequently, applicants submit that the rejection of claims 18-24, and 29 under 35 USC § 103(a) over Spitsbergen, Driever, Cheng, and Alexander in view of Epstein is improper and should be withdrawn.

Claim 25 was rejected under 35 USC § 103(a) as being unpatentable over Cheng and Spitsbergen, in view of Vogelstein et al. (U.S. Patent No. 6,511,818).

Vogelstein does not teach combining the haplotype screen with the cancinogenesis screen to make mutation search more efficient and targeted and to allow cloning of only genes that are phenotypically known to be involved in both cell proliferation and carcinogenesis. Therefore, Vogelstein does not overcome the deficiency in Cheng and Spitsbergen as described above, the discussion of which is incorporated herein by reference. Consequently, applicants submit that the rejection of claims 25 under 35 USC § 103(a) over Cheng and Spitsbergen in view of Vogelstein is improper and should be withdrawn.

Claims 26 and 27 were rejected under 35 USC § 103(a) as being unpatentable over Cheng and Spitsbergen in view of O'Reilly.

Applicants strongly disagree. Spitsbergen and Cheng were discussed above and the arguments are incorporated herein. Like teachings of Epstein, wherein only specific marker is taught, O'Reilly only teaches TUNEL as a method for identifying cell proliferation defects. And again, O'Reilly does not teach the step of combining the two methods to target the "mutation hunt" only to cloning of genes that are relevant. Thus, O'Reilly does not overcome the deficiencies in the four other cited references. Like for the arguments discussed above, the examiner also here has provided no indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness.

Therefore, applicants submit that the rejection of claims 26 and 27 under 35 USC § 103(a) over Cheng and Spitsbergen, in view of O'Reilly is improper and should be withdrawn.

Claim 28 was rejected under 35 USC § 103(a) as being unpatentable over Cheng and Spitsbergen, in view of Li.

Applicants again strongly disagree. Cheng and Spitsbergen were discussed above and the arguments are incorporated herein. Li teaches that BrdU stain can be used as a diagnostic marker for tumors but this teaching does not overcome the deficiencies in the four other cited references. Like O'Reilly, Li does not teach combining the haploid screen and carcinogenesis screen to make carcinogenesis affected gene/mutation hunt more targeted. Therefore, as discussed above, Li does not overcome the deficiencies in the other references. Once again, the examiner fails to provide any indication that one of ordinary skill in the art, without knowledge of the claimed invention, would piece together the teachings of these references to result in the claimed invention. The only way to achieve such result is through impermissible hindsight obviousness. Therefore, applicants submit that the rejection of claim 28 under 35 USC § 103(a) over Cheng and Spitsbergen, in view of Li is improper and should be withdrawn.

In conclusion, one skilled in the art, without knowledge of the present invention and thus without the benefit of impermissible hindsight, would not have been motivated to combine the primary reference discussing the haploid screen which was indicated to be not a good method for identifying traits that develop later in fish life, because the embryos die early due to the haploid genome, with a carcinogenesis screen, which is a long term screen of a fish itself.

In view of the foregoing, applicant respectfully submit that all claims are now in condition for allowance.

Applicants herein petition for extension of time for three months and herewith the Commissioner is authorized to charge the Nixon Peabody Deposit Account 50-0850 for the requisite extension fees associated with this submission. In the event that any additional fees are required, the Commissioner is authorized to charge Nixon Peabody deposit account No. 50-0850 for fee deficiencies associated with this submission.

Respectfully submitted,

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David S. Resnick, Reg. No. 34,235
Leena H. Karttunen (37 CFR 10.9(b))
NIXON PEABODY LLP
101 Federal Street
Boston, MA 02110
(617) 345-6054/1367 (phone)
(866) 743-2115 (fax)